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Seroepidemiology of New AIDS-Associated Adenoviruses Among the San Francisco Men's Health Study

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A seroprevalence survey to recently proposed adenovirus (AV) serotypes AV 48 and AV 49, isolated primarily from AIDS patients, was conducted among the San Francisco Men's Health Study cohort. This cohort of homosexual, heterosexual, or bisexual HIV-seronegative and -seropositive men from selected San Francisco census tracts has been studied since 1984. The presence or absence of type-specific antibody in 628 serum specimens from 1989 was determined by microneutralization. Thirty of these subjects (26 positive and four negative) were studied longitudinally. Serum specimens taken at 6-month intervals from 1984 to 1993 were tested to characterize antibody response and to document the advent of these new serotypes. Eight subjects were tested against five other AV serotypes for comparison. AV 48 and AV 49 seroprevalence rates were significantly higher in HIV-seropositives, but infection was not limited to the immunocompromised. Sexual preference was not a significant determinant for AV seroprevalence in HIV-seronegatives. However, the extent and duration of the neutralizing antibody response was strikingly different between homosexuals and heterosexuals: an endemic pattern of continuous reexposure over the 9-year period was seen in 90% of 19 homosexuals, while five of six heterosexuals (83%) had an episodic pattern of exposure with antibody decline to undetectable levels. These data suggest that these viruses may be endemic in some part of the homosexual population and that sexual transmission may be the primary source of continuous reexposure.

KEY WORDS: sexual transmission, adenovirus type 48, adenovirus type 49, adenovirus epidemiology

INTRODUCTION

Human adenoviruses (AVs) are causative agents of acute respiratory disease, epidemic keratoconjunctivitis, pneumonia, gastroenteritis, and severe systemic infections, particularly in the immunocompromised, including persons with AIDS [Flomberg et al., 1987; Hierholzer et al., 1988; Schnurr et al., 1995; Schnurr and Dondero, 1993; Wadell, 1990; Wigand and Adrian, 1986]. In 1988 five new subgenus D AV serotypes, isolated from AIDS patients primarily in the New York area, were identified and designated AV 43 to AV 47 [Hierholzer et al., 1988]. More recently, two additional subgenus D serotypes, isolated almost exclusively from AIDS patients in the San Francisco Bay area and Los Angeles, have been proposed as serotypes AV 48 and AV 49 [Schnurr and Dondero, 1993]. To date, 13 strains related to AV 48 and eight related to AV 49 have been found in California, isolated from a wide spectrum of clinical presentations, from pneumonia to encephalitis. The earliest isolates of AV 48 were found in 1985, and the peak number occurred in 1987. The earliest and maximum number of AV 49 isolates occurred in 1986, except for a single cross-reactive intermediate found retrospectively among untypable isolates from 1983. Only in one case was AIDS unequivocally not a factor.

The observation that the last seven new serotypes are found almost entirely in AIDS patients raises a number of questions. One concerns the apparent limitation to the immunocompromised. This limitation may be real: subgenus B serotypes AV 34 and AV 35 are rarely isolated from the immunologically competent [Flomenberg et al., 1987]. However, infections in the community may be subclinical so that they are only apparent in those with underlying disease. The limitation to AIDS patients may also be a function of route of transmission. Early studies on AV transmission defined two distinct path-

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TABLE I. AV 48 and 49 Serostatus vs. HIV Serostatus, SFMHS, Wave 10, 1989 (n = 628)

	HIV serostatus	
	+ (n = 268)	− (n = 360)
AV +	24.6%[a] (64/268)	10.5%[a] (37/360)
AV 48	51% (33/64)	46% (17/37)
AV 49	22% (14/64)	30% (11/37)
Both	27% (17/64)	24% (9/37)
	$P < 0.0005$[a]	

[a]χ^2 test.

TABLE II. Longitudinal Mean Titers* to AV 2 and AV 7, SFMHS Waves 1–18, 1984–1993 (n = 8)

	AV 2	AV 7
HIV-seronegatives		
Homo/bisexual (n = 4)	292.5 (4/4)	39.2 (3/4)
Heterosexual (n = 2)	131.1 (2/2)	19.6 (2/2)
HIV-seropositives (positive at entry, n = 2)	203.5 (2/2)	32.5 (2/2)
Seroprevalence[a]	100%	88%

*Longitudinal mean titers are calculated as (1) the mean over time for each patient, then (2) the mean of all patients within each category.
[a]In any specimen.

ways. Among adults transmission occurs by respiratory droplet, but among young children fecal–oral spread predominates. Even in respiratory disease, AVs are shed from the colon in greater numbers and for a longer period of time than from the respiratory tract, and asymptomatic persistent shedding of virus for weeks to months is not uncommon [Fox and Hall, 1980]. AVs have been identified as sexually transmitted agents, implicated in nongonococcal, nonchlamydial urethritis in men [Harnett and Newnham, 1981; Harnett et al., 1984; Swenson et al., 1995], in male sterility, and in recurrent abortion in women [Kulcsar et al., 1991]. Homosexual transmission, combining aspects of heterosexual transmission with fecal–oral transmission, may play a significant role in the dissemination of these viruses. It is possible that there is a reservoir of infection within the homosexual community that puts persons with AIDS at a higher risk of infection. This has been demonstrated for several gastrointestinal pathogens causing diarrheal disease in homosexual men [Janoff and Smith, 1988].

Neutralizing antibody response to AV infection is type-specific. Early studies showed that neutralizing antibody appeared within 2 weeks of primary infection, at relatively low titers of 8 to 80, then declined to undetectable levels over a 2-year period if reexposure did not occur [Jawetz et al., 1957]. Seroprevalence and antibody levels to any given serotype, therefore, depended on the levels of that virus circulating in the community. Seroprevalence to the endemic serotypes were from 25% to 82%, but only from 0% to 6% against the serotypes involved in sporadic outbreaks of keratoconjunctivitis [Fox and Hall, 1980; Huebner et al., 1954; Tai and Grayston, 1962].

Here, we report the results of a serologic survey for neutralizing antibody to AV 48 and AV 49 among HIV-seronegative and -seropositive homosexual, bisexual, and heterosexual men from San Francisco. Our objectives were (1) to establish a seroprevalence rate among HIV-seropositives and -seronegatives to determine whether infection is associated with impaired immune function, (2) to examine whether sexual orientation played a role in infection rates, (3) to document the appearance of these viruses by the earliest dates of infection, and (4) to characterize the neutralizing antibody responses to these two viruses.

MATERIALS AND METHODS

Study Population

The San Francisco Men's Health Study (SFMHS) was a cohort of single men aged 25 to 55 at the time of enrollment in late 1984. Subjects were selected randomly, by block and by household, from 19 census tracts in San Francisco with the highest seroprevalence to HIV. The original cohort was composed of 1,034 men, approximately 800 homosexual and bisexual men and 200 heterosexuals. At the time of entry, 48.6% of the homo/bisexual men were HIV-seropositive [Winkelstein et al., 1987]. Sequential data and specimen collection continued at 6-month intervals until 1993. In any one 6-month period (designated a wave) not all members responded and since 1984 223 men have died of AIDS, so the demographic parameters of any one wave varied slightly. In Phase 1 of this study, seroprevalence to AV 48 and AV 49 in a set of specimens from 1989 was determined and the data were analyzed by demographic characteristics. In this set, there were 628 respondents, 43% seropositive for HIV, 524 homo/bisexual men, 99 heterosexual men, and 35 for whom sexual orientation information was unavailable. Phase 2 was a quantitative longitudinal study of AV 48 and 49 antibody levels on 30 selected subjects, from 1984 to 1993. Selection was based on demographics so that all groups were represented: 10 HIV-seropositive homosexual men, 12 HIV-seronegative homo/bisexual men, and eight HIV-seronegative heterosexual men; 26 were AV 48- and/or AV 49-seropositive and four AV-seronegative. A subset of eight of these 30 were tested for neutralizing antibody to five other AV serotypes to provide context and a basis for comparison of endemic vs. sporadic patterns of response in this population: endemic serotypes AV 2 and AV 7; transplantation-associated AV 35, isolated only from the immunocompromised [Flomenberg et al., 1987]; and AV 44 and AV 47, isolated from AIDS patients in the eastern United States [Hierholzer et al., 1988].

Neutralizing Antibody Determination

A semiautomated colorimetric microtiter neutralization assay for AVs was used to assess type-specific neutralizing antibody [Crawford-Miksza and Schnurr, 1994]. The extent of cell destruction was assessed at A_{550} in a spectrophotometer. Antiserum neutralization at each dilution was calculated as a percentage of normal cell control minus the virus control (percent neutraliza-

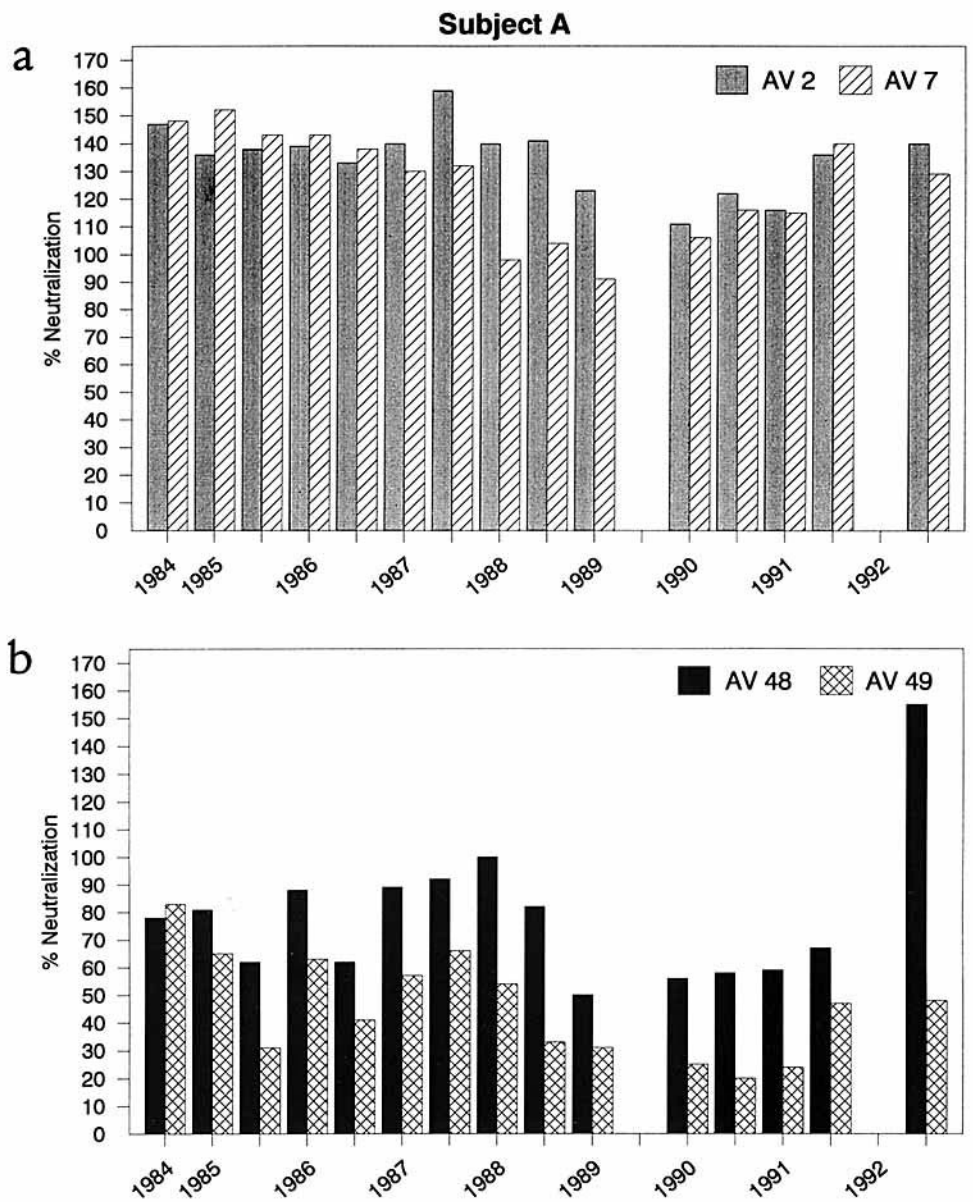

Fig. 1. Longitudinal profile of AV antibody in subject A (HIV-seronegative, homosexual). Subject was not seen at waves 11 (1989), 16 (1992), and 18 (1993). Percent neutralization is the fraction of virus neutralized by subject serum at a given dilution as compared to cell and virus controls. **a:** Endemic pattern of exposure to AV 2 and AV 7. **b:** Endemic pattern of exposure to AV 48 and AV 49.

tion), as an average of four replicate wells per serum and virus dilution, and cell control. In Phase 1, sera were screened at a single dilution and designated positive if they reduced cytopathic effect (CPE) by >50% over virus controls. In Phase 2, sera were serially diluted beginning at 1:10, and percent neutralization was determined. Titers were calculated using a 50% neutralization endpoint. All specimens from a given subject were tested in the same assay. Stock viruses were prepared as previously described [Crawford-Miksza and Schnurr, 1994] from prototype strains from the collection of the Viral and Rickettsial Disease Laboratory, California Department of Health Services (Berkeley, CA). A single preparation of each stock virus was used in all tests. Standardized prototype antisera to each serotype were included in each assay as controls.

RESULTS

Phase 1

A total of 16.1% of the 628 subjects had neutralizing antibody to AV 48 or AV 49 or both. The seroprevalence rate for AV 48 was 12.1% (76/628), for AV 49 8.1% (51/628), and for both 4.1% (26/628). Table I shows the data sorted for HIV seroprevalence vs. AV seroprevalence. There was a significantly higher rate among HIV-seropositives, more than twice that for HIV-seronegatives.

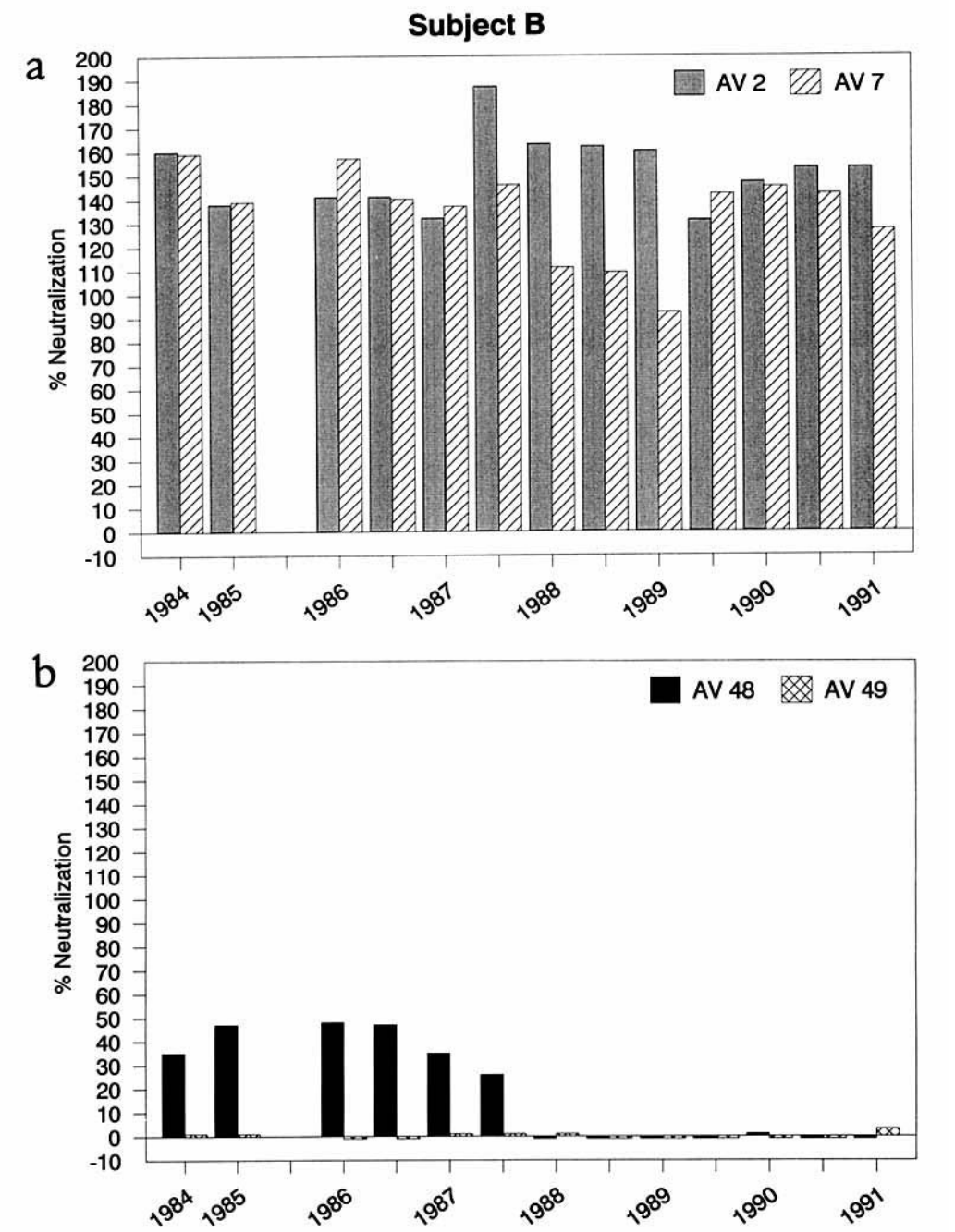

Fig. 2. Longitudinal profile of AV antibody in subject B (HIV-seronegative, heterosexual). Subject was not seen at waves 3, 15–18. **a:** Endemic pattern of exposure to AV 2 and AV 7. **b:** Episodic pattern of exposure to AV 48.

Distribution of AV-seropositives within each category for one or the other or both viruses was not significantly different, approximately half positive for AV 48, a quarter positive for AV 49, and a quarter positive for both.

The analysis of AV serostatus vs. sexual orientation included only HIV-seronegatives since the higher rate among HIV-seropositives, all of whom were homosexual, might affect results. Seroprevalence among homo/bisexual men was 12.4% (28/226) and 8.2% (8/98) among heterosexuals, which was not significantly different.

Data were also analyzed geographically, by census tract, for HIV seroprevalence, sexual orientation, and AV seroprevalence, using χ^2. While sexual orientation varied significantly between census tracts ($P < 0.0005$) and HIV seroprevalence variation was almost significant ($P < 0.075$), AV seroprevalence was fairly evenly distributed geographically ($P < 0.4$).

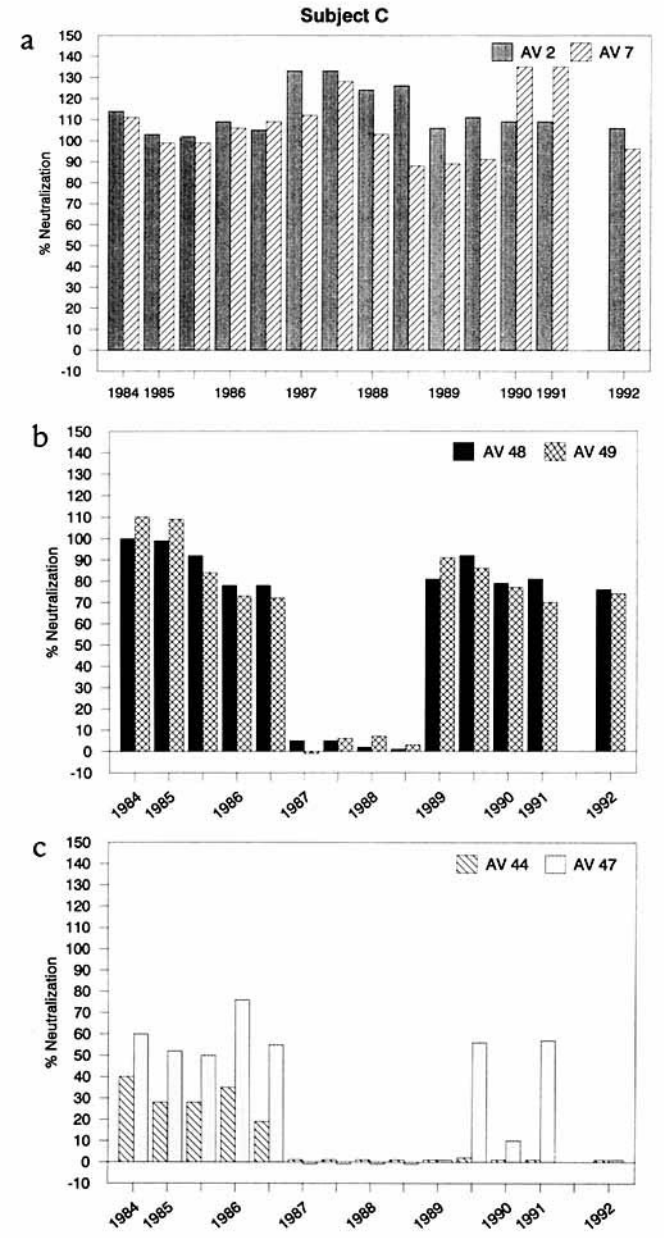

Fig. 3. Longitudinal profile of AV antibody in subject C (HIV-seroconverter, homosexual). Subject was not seen at waves 14 and 16–18. **a:** Endemic pattern of exposure to AV 2 and AV 7. **b:** Endemic pattern variation to AV 48 and AV 49 with loss of antibody from 1987–1988. **c:** Antibody to AV 44 and AV 47, also lost from 1987 to 1989. Seroconverted to HIV in 1989.

Phase 2

Table II summarizes seroprevalence and antibody titers in a representative sample of eight men over the 9 years of observation to AV 2 and AV 7. High levels of antibody were present for the entire period, in eight of eight individuals to AV 2 and in seven of eight to AV 7, consistent with an endemic pattern of continuous reexposure. This pattern is illustrated for three individuals in Figures 1a, 2a, and 3a. Longitudinal mean titers to AV 2 and AV 7 were not significantly different between categories (Table II) and were approximately 10-fold higher to AV 2 than AV 7. None had antibody to AV 35. Antibodies to AV 44 and AV 47 were seen in five of eight and four of eight, respectively, indicating that these viruses were in circulation on the West Coast as well.

Table III summarizes longitudinal seroprevalence and mean titers to AV 48 and AV 49. Significant differences between categories become evident here and are illustrated in Figures 1b and 2b. Among the homo/bisexual men, 80% of HIV-seronegatives and 100% of HIV-seropositives had neutralizing antibody to AV 48, AV 49, or both in 75% or more of their specimens over the entire period from 1984 to 1993, consistent with continuous reexposure (Fig. 1b). In contrast, five of six heterosexuals (83%) demonstrated an antibody response of limited extent and duration (Fig. 2b), with complete antibody decay within 1–3 years, consistent with a single episode of infection without reexposure. Within this group, the episodes were scattered throughout the 9-year study period with no discernible point source outbreak. These differences were reflected in longitudinal mean titers, which were significantly different between categories for AV 48 but not AV 49. A single heterosexual, HIV-seronegative subject had antibody to AV 49 over the whole period, and his longitudinal average titer of 40 raised the whole group mean.

A variation of the endemic pattern was seen in three HIV-seronegative, homosexual men and in one HIV seroconverter. Antibody to AV 48 and AV 49 disappeared completely over a 2-year period from 1987 to 1988, only to reappear at previous levels in 1989 (Fig. 3b). The pattern was nearly identical in all four subjects within the same time frame. Antibody to AV 44 and AV 47 also disappeared during this interval in the one subject for whom this was tested (Fig. 3c). Antibody levels to AV 2 and AV 7 remained high during this same 2-year period (Fig. 3a). These results were confirmed in several repetitions of the assays.

DISCUSSION

Infections with AV 48 and AV 49 are not limited to HIV-seropositives or HIV-immunocompromised subjects, as these viruses are capable of infecting and eliciting a neutralizing antibody response in the general population at seroprevalence levels intermediate between those for endemic and sporadic serotypes. The higher seroprevalence rate in HIV-seropositives may be accounted for in several ways. It may be due to an increased susceptibility requiring a lower virus dose to establish infection or the establishment of persistant infection in an immunocompromised population. It also may be due to HIV-induced polyclonal B-cell activation that results in high levels of antibody to many different antigens [Levy, 1993]. Alternatively, it may represent a true difference in exposure to sexually transmitted pathogens, including HIV.

It was hoped that an earliest date of infection with these viruses could be documented and that it would be possible to track the infection from one group to another. That has proved to be impossible with this cohort. These

TABLE III. Cumulative Seroprevalence and Longitudinal Mean Titers to AV 48 and 49 Among Selected AV-Seropositives, SFMHS Waves 1–18 (n = 25)

	AV 48 and AV 49 antibody in >75% of specimens	Longitudinal mean titer (all specimens)	
		AV 48	AV 49
HIV-seronegatives			
Homo/bisexual (n = 10)	80% (8/10)[a]	24.4 (8/10)[b]	8.9 (7/10)[c]
Heterosexual (n = 6)	17% (1/6)[a]	1.5 (6/6)[b]	11.0 (3/6)[c]
HIV-seropositives (positive at entry, n = 9)	100% (9/9)	105.1 (9/9)	16.4 (5/9)
	$P < 0.01$[a]	$P < 0.025$[b]	NS[c]

[a]χ^2 test.
[b]t test.
[c]Not significant. A single heterosexual subject had a longitudinal mean titer of 40. When this anomaly was excluded, the longitudinal mean titer for that category was 1.3, which meets the criterion for significance ($P < 0.05$).

viruses were well established in this community a year and a half or more before the first strains were isolated. From the geographic and seroprevalence data, both viruses appear to have been distributed throughout the San Francisco community.

Two distinct patterns of antibody response to AV 48 and AV 49 emerged from the longitudinal studies. An endemic pattern that is associated with continuous reexposure was seen in 90% of 19 homosexual men. When the possible effects of HIV infection increasing antibody levels are discounted, this pattern was present in 80% of 10 HIV-seronegative homosexual men. Longitudinal mean titers to AV 48 were comparable to those of endemic serotype AV 7 for this group. In contrast, 83% of heterosexual men had a low-level antibody response for a short period of time, characteristic of sporadic exposure. The differences in extent and duration of antibody response between these groups were statistically significant.

AV 2 and AV 7 were selected for the panel of other AVs because they are the serotypes that have been isolated most frequently over the last 5 years in this laboratory [L.C.-M., personal observation]. An endemic pattern to AV 2 and AV 7 was seen in all categories of subjects in this sample. This is confirmation of earlier observations that continuous reexposure by respiratory droplet to endemic serotypes is characteristic of adult AV transmission [Fox and Hall, 1980; Huebner et al., 1954; Tai and Grayston, 1962].

The association of an endemic pattern of response and homosexual orientation for AV 48 and AV 49 suggests that homosexual transmission is the primary source for continuous reexposure. It is probable that maintenance of antibody over 8–9 years in the homosexual subjects represents some reservoir of viral shedding among sexual contacts within the community. Persistent infection with extended viral shedding from the colon for up to 3 years is well documented for subgenus C AVs [Fox and Hall, 1980; Wadell, 1990; Wigand and Adrian, 1986] but has not been reported previously for subgenus D AVs in the immunocompetent.

None of the subjects tested had antibody to AV 35. Although this serotype is frequently associated with infections in the immunocompromised, those infected rarely produce an antibody response [Horwitz et al., 1884].

The loss of antibody seen in the variation of the endemic pattern in three HIV-seronegative subjects and one seroconverter suggests that they were not exposed to these agents for approximately 4 years, from 1985 to 1988, given an average 2-year rate of decline of antibody to undetectable levels [Jawetz et al., 1957; Tai and Grayston, 1962]. In contrast, antibody levels in these same subjects to endemic serotypes AV 2 and AV 7, usually transmitted by the respiratory route, remained high during this same period. Winkelstein et al. [1987] reported a 60% reduction of high-risk sexual behavior in this cohort from 1984 to 1986, which resulted in reduction in HIV transmission. In this study, 16% of positive subjects demonstrated a loss of antibody during this period. Loss of antibody to AV 48 and AV 49 due to lack of exposure during this time may be due to the reported behavioral modification. The return to high antibody levels in 1989 may represent a return to high-risk behavior, which is strengthened by the fact that one of the four seroconverted to HIV at that point.

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